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Fatty acids Analyses of n-Hexane Fractions of Ageratum conyzoides Leaf

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Abstract
Lipidomics is an emerging field, where the structures, functions and dynamic changes of lipids in cells, tissues or body fluids are investigated. This study revealed the GC–MS metabolic profiling of the polar and non-polar fractions from n-hexane extracts of *Agerantum conyzoides.* After extraction, the n-hexane leaf extract has a yield of 1.20% and the GC-MS result reveal that *A. conyzoides* have ω-6: ω-3 PUFA ratio of 2.1 and other fatty acids of biochemical relevance.
Keywords: Lipidomics; Cardiovascular disease; *Agerantum conyzoides* leaf; Fatty acids, GC-MS.

Introduction

Ageratum conyzoides (Goat weed) is an erect softly hairy herbaceous annual herb ramified and up to 1 m tall (Nyunai *et al*., 2010). It was originally introduced as a garden plant, and now widely used in traditional medicine worldwide (Kamboj and Saluja, 2008). Since ancient times, the plant had been utilized for the treatment of various ailments, such as burns and wounds, infectious diseases, arthritis and fever constipation, hepatitis, eczema, epilepsy, dizziness, diarrhoea, vomiting, fever, headaches, intestinal worms and filariasis. An ethno-botanical study reported that, leaves, or entire plant decoction is useful for the treatment of diabetes. The hypoglycaemic and anti hyperglycaemic properties of the extracts of the leaves of *A. conyzoides* have being validated in normal and hyperglycaemic rats (Nyunai *et al*., 2010). It has been assumed that "there is a plant for every need on every continent". Remarkably, this statement appears to be true (Omotoyinbo and Sanni, 2015).

Cardiovascular disease (CVD) is one of the most common in the Western world; its complex aetiology involving both genetic and acquired factors. Public health messages about diet and CVD have tended to focus on saturated fatty acids (SFA) and ω-6 PUFA. More recently, the messages have included the positive role of ω-3 PUFA (Ruxton *et al*., 2004). The lower rate of CVD seen in fish-eating communities prompted investigation into how fish and its nutritional components might lower the risk of CVD (Bulliyya, 2002).

Poly unsaturated fatty acids (PUFAs) can be classified as either ω-3 (omega 3) or ω-6 (omega 6) PUFAs; to which family a PUFA belongs depends on the position of the first double bond in the fatty acid chain. These fatty acids had been established to be responsible for the Low density lipoprotein cholesterol (LDL-C) and total cholesterol reducing potential (Ibukun and Oladipo, 2016). High density lipoprotein (HDL) cholesterol is the heart-friendly lipoprotein that counters the action of LDL by removing cholesterol from the arteries and transporting it back to the liver for safe disposal (Colpo, 2005). This study will evaluate the fatty acids constituents of *Ageratum conyzoides* leaf hexane extract.

Material and Methods

Sample collection

The aerial parts of *Ageratum conyzoides* was collected from farm garden located in Ilara-Mokin, Ondo State (Nigeria). The plant was identified in Crop Soil and Pest Management depatment, FUTA.

Method of extraction

After authentication, the aerial parts of *A. conyzoides* at flowering stage were collected in bulk, washed under running tap water to remove adhering dirt, followed by rinsing with distilled water, air dried in shadow and grinded by mixer grinder. After grinding, 150 g of the dried leaf was soaked in 0.6 liters of n-hexane at 40˚C to 45˚C for 6 hours. The organic solvent was filtered through Cheese cloth and filter paper (whatman) till clear solution was obtained. Solvent was evaporated in a rotatory evaporator (Buchi, Switzerland) under reduced pressure (vacuum) at 40˚C and concentrated. The crude extract was stored in air tight container at dark place (Ruedeekorn *et al.,* 2009).

Fatty acid methyl ester analysis

50mg of the extracted fat content of the sample was saponified (esterified) for five minutes at 95^{o}C with 3.4ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl. 3ml of the 14% boron triflouride in methanol was added. The mixture was heated for 5 minutes at the temperature of 90^{o}C to achieve complete methylation process. The fatty acid methyl esters were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1ml for Gas Chromatography analysis and 1μl was injected into the injection port of GC. The GC equipment used was HP 6890 powered with HP chemstation Rev.

A09.01 (1206) software. The split ratio was 20:1, the carrier gas was nitrogen at inlet temperature of 250^{0}C with a column type of HP INNOWax and column dimensions of 30m x 0.25mm x 0.25μm. The oven program parameters include initial temperature at 60^{o}C, first ramping at 12^{o}C/minutes for 20 minutes, maintain for 2 minutes and second ramping at 15^{o}C/min for 3minutes, maintained for 8minutes. The detector used was FID at 320^{o}C at hydrogen pressure 22psi and compressed air of 35psi.

Results and Discussion

The qualitative and quantitative estimation of fatty acids in the samples revealed the following fatty acids in g/100g of samples; Caprylic acid (C8:0); 0.0032g, Capric acid (C10:0); 0.0026g, Lauric acid (C12:0); 0.008g, Myristic acid (C14:0); 0.8082g, Palmitic acid (C16:0); 37.115g, Palmitoleic acid (C16:1); 1.1488g, Margaric acid (C17:0); 0.048g, Stearic acid (C18:0); 5.503g, Oleic acid (C18:1); 6.142g, Linoleic acid (C18:2); 32.44g, Linolenic acid (C18:3); 15.45g, Arachidonic acid (C20:4); 0.053g, Arachidic acid (C20:0); 0.579g, Behenic acid (C22:0); 0.427g, Erucic acid (C22:1); 0.212g, and Lignoceric acid (C24:0); 0.0643g.

The roles of PUFAs become more important as they are not synthesized in human organism and have to be delivered with food (Ibukun and Oladipo, 2016). SFAs with chain lengths longer than 10 carbons generally raised blood cholesterol levels; PUFA (primarily linoleic acid) lowered blood cholesterol levels; and MUFA (primarily oleic acid) had either a neutral or mildly hypocholesterolemic effect on blood cholesterol levels. SFA stearic acid did not raise blood total or LDL cholesterol (i.e., the “bad” cholesterol) levels (Mensink, 2005). Lauric, myristic, palmitic acids and stearic acid had been shown to exert a neutral or hypocholesterolemic effect on blood cholesterol levels in experimental animals (Kris-Etherton *et al.,* 2005). Caprylic acid has antibacterial, antiviral and antifungal properties and it can help treat health problems associated with the overgrowth of yeast, such as vaginal yeast infections, candida, and thrush. It also helps to normalize the acidity stomach to its normal levels. In addition, caprylic acid is purported to manage high blood pressure, treat Crohn's disease, as well as reduce cholesterol levels and fight off bacterial infections. At high concentrations of linoleic acid, conversion of α-linolenic acid to eicosapentaenoic and docosahexaenoic acids is inhibited. Most probably, the high proportion of linoleic acid to linolenic acid in organism negatively influences the deficiency of long-chain fatty acids (Steinhart *et al.,* 2003).

The consumption of a solid fat rich in lauric acid would result in a more favorable blood lipid profile than the consumption of a solid fat rich in trans-fatty acids. Stearic acid does not seem to raise low-density-lipoprotein cholesterol relative to oleic acid, which is known to be neutral in its effects on cholesterol concentrations. In contrast, palmitic acid, another long-chain saturated fatty acid, raises cholesterol concentrations. Palmitoleic acid is a monounsaturated fatty acid resembling saturated fatty acids in its ability to lower LDL-C (Nestel *et al,* 1994).

The qualitative and quantitative estimation of fatty acids in the samples revealed the fatty acids using Gas Chromatography Mass Spectrophotometer. The n-hexane extracts contained ω-3 fatty acid (PUFA) precursor, α-linolenic acid (15.446). The ω-6 PUFA present are linoleic acid (32.440) and arachidonic acid (0.053). Although the overall result revealed that ω-6 PUFAs have a total content of 32.493g per 100g of oil extracts while ω-3 PUFAs have a total content of 15.446g per 100g oil extracts, bringing the ratio of ω-6:ω-3 to 2.1. *A. conyzoides* leaf had very low concentration of Capric acid (0.003) and with predominant amount of Palmitic acid (37.115g per 100g) of n-hexane extract. According to the World Health Organization, the consumption of palmitic acid increases risk of developing cardiovascular diseases (WHO, 2003). Rats fed a diet of 20% palmitic acid and 80% carbohydrate for extended periods showed alterations in central nervous system control of insulin secretion, and suppression of the body's natural appetite-suppressing signals from leptin and insulin (Benoit *et al.,* 2009).

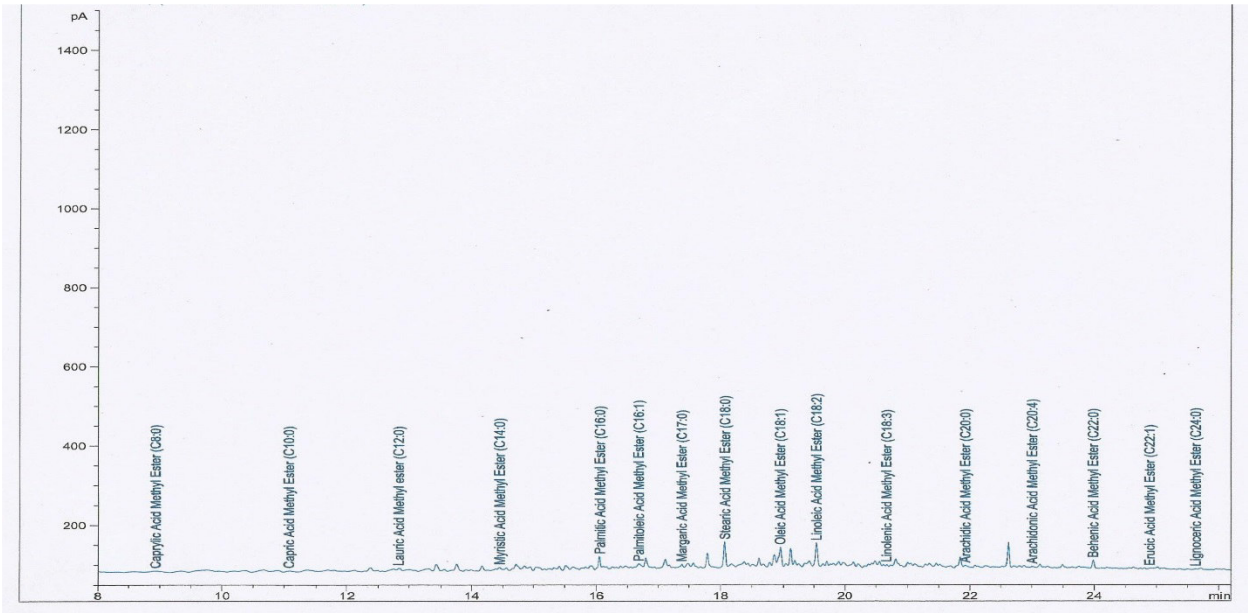

Figure 1: GC-MS Profile of n-hexane leaves extract of *Ageratum conyzoides.* Ratio of n-6: n-3 is 2.1

The fatty acids composition of the *A. conyzoides* leaf; the ω-6:ω-3 ratio can be inferred to be considerably responsible for the antioxidant and anti-inflammatory activities of the leaf. It is supposed that ω-3 and ω-6 polyenic fatty acids are metabolized by identical enzymatic systems (Małgorzata *et al.*, 2005).

Researchers at different circumstances had recommended different ratios of the PUFA which included; 1:1 (Andrew, 2001), 5:1, 4:1 and 10:1 (Allport, 2007). The rationale for the proposed ratios relates to the belief that the two classes of fatty acids interact within the human body. ω-3 and ω-6 fatty acids follow parallel pathways, continually competing with each other for chemical conversion to various structures and molecules inside and outside the cells. Given this mechanism, it makes sense that the two fatty acids might be required in approximately equal amounts (Andrew, 2001).

Conclusion

The study had been able to establish *Ageratum conyzoides* leaf as having lipidomics dietary relevance; the fatty acids composition of n-hexane leaf extract of *Ageratum conyzoides* as having CVD alleviating potency, justified by the amount of omega-3 fatty acids and the ratio of the omega-6:omega-3 values thus establishing the extract as dietary therapy for alleviating benign and malignant growths. The normo-cholesterolemic activity of the leaf extract is verified by the negligible amount of cholesterol reducing Capric acid.

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